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Expression of a human cytomegalovirus gp58 antigenic domain fused to the hepatitis B virus nucleocapsid protein

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Abstract

Hepatitis B virus core antigen (HBcAg) has been used as a carrier for expression and presentation of a variety of heterologous viral epitopes in particulate form. The aim of this study was to produce hybrid antigens comprising HBcAg and an immunogenic epitope of human cytomegalovirus (HCMV). A direct comparison was made of amino and carboxyl terminal fusions in order to investigate the influence of position of the foreign epitope on hybrid core particle formation, antigenicity and immunogenicity. HCMV DNA encoding a neutralising epitope of the surface glycoprotein gp58 was either inserted at the amino terminus or fused to the truncated carboxyl terminus of HBcAg and expressed in *Escherichia coli*. The carboxyl terminal fusion (HBc_{3-144}–HCMV) was expressed at high levels and assembled into core like particles resembling native HBcAg. Protein with a similar fusion at the amino terminus (HCMV–HBc_{1-183}) could not be purified or characterised immunologically, although it formed core like particles. HBc_{3-144}–HCMV displayed HBc antigenicity but HCMV antigenicity could not be detected by radioimmunoassay or western blotting using anti-HCMV monoclonal antibody 7-17 or an anti-HCMV human polyclonal antiserum. Following immunisation of rabbits with HBc_{3-144}–HCMV, a high titre of anti-HBc specific antibody was produced along with lower titres of HCMV/gp58 specific antibody.

Keywords: Prokaryotic expression; Fusion protein; Hepatitis B core antigen; Human cytomegalovirus; Envelope glycoprotein

1. Introduction

The use of HBcAg to present the foreign epitopes to the immune system offers several advantages. It can be expressed in a wide range of systems, including bacterial [1,2], insect [3], mammalian [4] and yeast [5] cells and recombinant vaccinia viruses [6]. In addition, HBcAg is highly immunogenic, acting as a T-cell independent and T-cell dependent antigen [7]. Furthermore, both the amino [6,8] and carboxyl [9,10] termini of HBcAg have been used separately to express several fusion proteins.

The amino terminus of HBcAg has been fused to amino acids 141–160 of the VP1 protein of foot and mouth disease virus (FMDV) serotype O_1. Although the recombinant protein proved to be toxic for *Escherichia coli*, it has been expressed efficiently using a vaccinia virus system to give rise to a highly immunogenic, particulate product [6]. Subsequently, an *E. coli* expression vector, pBC404 was developed with gene expression driven by the *tac* promoter [11]. Using this vector, an immunogenic epitope of

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poliovirus type 1 [11] and an epitope of the human rhinovirus type 2 capsid polypeptide VP2 [12] have been expressed in *E. coli* and found to be highly immunogenic. Similarly, heterologous amino acid sequences of variable length have been fused to a truncated carboxyl terminus and expressed as recombinant core particles in *E. coli* [9,10]. The coding sequences inserted in different constructs include those for preS1 (aa 1–20 and 1–36), preS2 (aa 109–134) and S (aa 111–156 and 111–165) epitopes of HBV. In addition, synthetic sequences coding for fragments of the transmembrane region (gp41) of the HIV-1 envelope protein have been expressed [9]. These fusion proteins were synthesised in *E. coli* yielding stable products which assembled to form particles morphologically similar to HBcAg. The proteins exhibit the immunogenic characteristics of the HBcAg epitopes and the epitopes carried by the additional sequences [9].

The amino and truncated carboxyl termini of HBcAg have been used separately for fusion of foreign epitopes in independent expression systems. Schödel et al. [13], used epitopes derived from preS1 and preS2 sequences of HBV and fused these in separate constructs to the amino and carboxyl terminus of HBcAg. Their results suggested that polypeptides fused to the amino terminus of HBcAg may not be expressed on the surface of core particles. The objective of this study was to compare the expression in *E. coli*, of a foreign sequence (a sequence encoding one of the neutralizing epitopes, gp58, of HCMV), fused to the amino or the truncated carboxyl terminus of HBcAg.

2. Materials and methods

2.1. Construction of expression plasmids

Amino terminal fusion: Plasmid p73HCMV comprises 149 bp of HCMV DNA encoding an immunodominant epitope of the surface glycoprotein gp58, derived from strain AD169 and cloned into the vector pT7T3/18U (Pharmacia Biotec, Uppsala). This HCMV sequence was amplified using the polymerase chain reaction (PCR) and primers which had *Eco*RI and *Bam*HI restriction enzyme sites incorporated at their 5′ ends (Fig. 1). The product was double digested with *Eco*RI/*Bam*HI, and ligated to similarly digested plasmid pPV404 [11], yielding plasmid pv404CMV. Poliovirus sequences in pPV404 were removed by the digestion with *Eco*RI/*Bam*HI. The integrity of the sequences across the junction sites in plasmid pv404CMV was confirmed by sequencing.

Carboxyl terminal fusion: HCMV sequences were amplified from plasmid p73HCMV using another primer pair with *Hin*dIII and *Bam*HI restriction enzyme sites at their 5′ ends (Fig. 1). The PCR product was double digested with *Hin*dIII and *Bam*HI and ligated into similarly digested plasmid ptacHpaII–R2 [9], forming recombinant plasmid ptacCMV. The integrity of the junction sites was confirmed by sequencing.

2.2. Protein purification

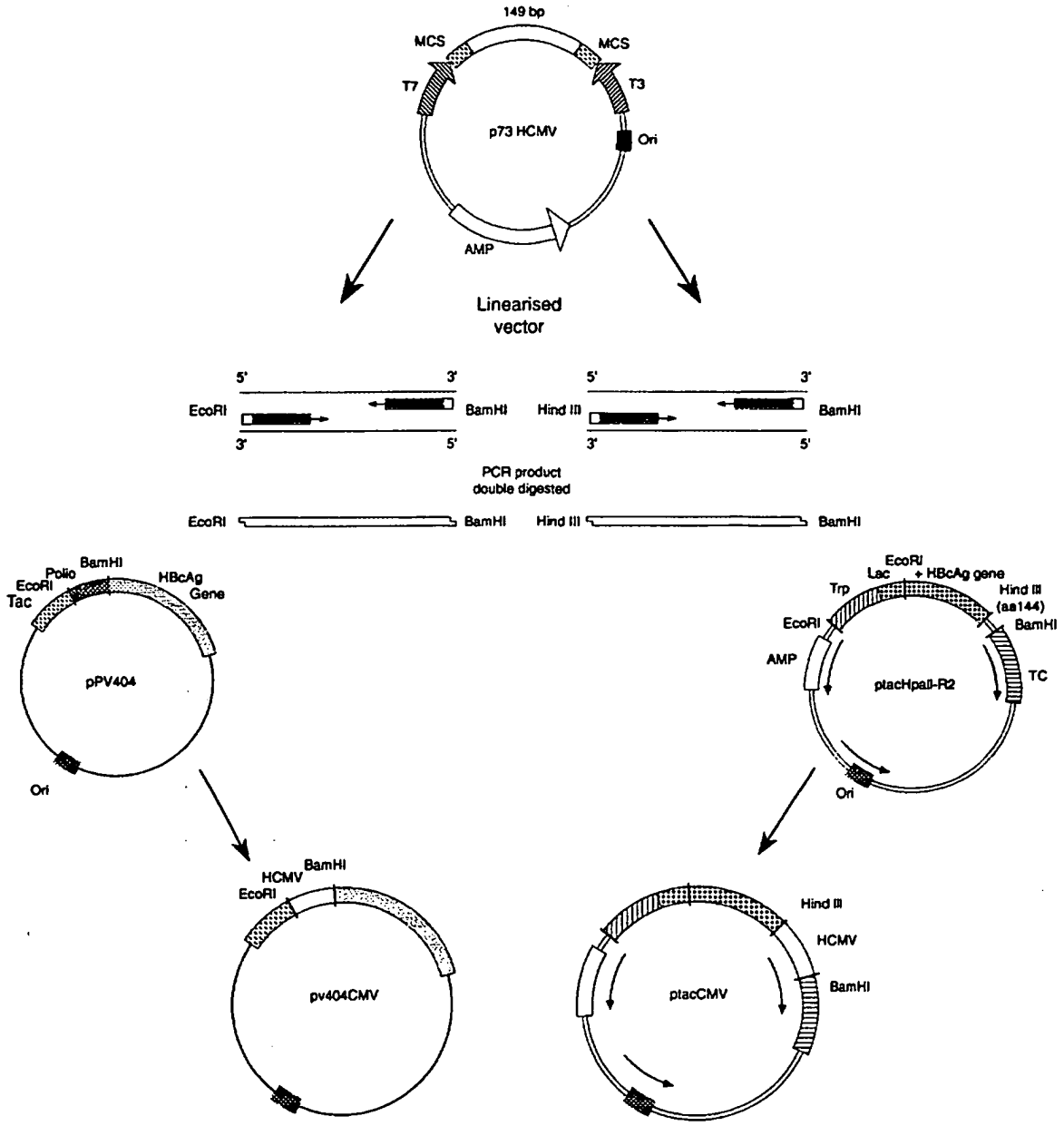
Cultures of *E. coli* strain RB791 (lac I^q) harbouring parental and recombinant plasmids were induced with IPTG and the proteins purified by chromatography on sepharose 4B–CL and ultracentrifugation [2,9]. The purified proteins were stained with Coomassie blue after resolution using SDS–PAGE and western blotting was carried out as described by Towbin et al. [14].

2.3. Immunisation of animals

The fusion protein (30 mg) was diluted in 0.5 ml PBS and 0.5 ml of complete Freund's adjuvant (CFA) for immunisation or the same volume of incomplete Freund's adjuvant (IFA) for booster injections. Three outbred Dutch male rabbits (6 months of age; 1.5 kg) were immunised intramuscularly with the fusion protein emulsified in CFA on day 0 and subsequently boosted with the fusion protein emulsified in IFA on days 30, 60 and 90. Blood was collected from rabbits on days 0 (preimmune; PI), 10 (first test bleed; 1TB), 40 (second test bleed; 2TB) 70 (third test bleed; 3TB) and 100 (fourth test bleed; 4TB).

2.4. Murine monoclonal antibodies

RFHBc17, the generous gift of Dr. J.A. Waters, is believed to recognise a major epitope of HBcAg and



Schematic representation of plasmid construction of ptacCMV and pv404CMV. MCS, multiple cloning site; T3&T7, phage promotors; AMP, ampicillin resistance gene; ori, origin of replication; Tac, tac promotor [▒]; polio, poliovirus sequences [▓]; Trp, Trp promoter [▒]; Lac + HBcAg gene, Lac promoter and hepatitis B core antigen gene [▒]; TC, tetracycline resistance gene [▒]. Position of some restriction enzyme sites are shown as black lines cutting across the plasmid.

Fig. 1.

reacts with native and recombinant (expressed in *E. coli*) antigen, but not with HBeAg [15]. Other anti-HBc monoclonals were the gift of Dr. E. Korec — 18C H_5G_1 and 18C $E_{11}B_{12}$, which react with native and recombinant HBcAg; 35H2, which reacts with HBeAg, as well as native and recombinant HBcAg; and A2C12, which reacts with HBeAg but not native or recombinant HBcAg [16,17].

Monoclonal anti-HCMV, 7-17, was the generous gift of Dr. W. Britt, University of Alabama at Birmingham, USA. This recognition site for this antibody is within the immunodominant AD-1 region of gp58 [18].

2.5. Radioimmunoassays (RIA) and immunofluorescence

To determine HBc and HCMV antigenicity of the fusion proteins, polystyrene beads coated with rabbit anti-HBc antibody were used in a modification of a sandwich RIA described previously [2]. A second antibody was then added; either mouse monoclonal anti-HBc, for HBc antigenicity assay, or human polyclonal HCMV antibody or mouse monoclonal anti-HCMV for HCMV antigenicity assay. Depending on the type of the second antibody used, further incubation was carried out with iodinated sheep anti-mouse or anti-human antibody.

Western blots were carried out following purification of recombinant proteins by ammonium sulphate precipitation and sepharose CL–4B chromatography. After separation on 12.5% SDS–polyacrylamide gels (without 2-mercaptoethanol), the polypeptides were transferred to nitrocellulose using standard methods.

A competitive radioimmunoassay was used to detect anti-HBc antibody in test sera, as described by Murray et al. [19]. Test serum dilutions (1 in 10, 1 in 30, 1 in 100) and positive and negative controls were incubated overnight with HBcAg coated beads in the presence of iodinated human anti-HBc IgG.

Anti-HCMV antibodies were detected in rabbit sera using immunofluorescence. Insect cells (Sf21) infected with a recombinant baculovirus expressing glycoprotein B (gB) of HCMV (the generous gift of Dr. W. Britt, University of Alabamha at Birmingham, USA) were fixed to glass slides. The cells were incubated with preimmune or immune rabbit sera at dilutions of 1 in 10, 1 in 20, 1 in 40, 1 in 60, 1 in 80 and 1 in 100. Uninfected Sf21 cells were used as a control. Only sera obtained from the third and fourth test bleeds were tested against HCMVgB-infected and uninfected Sf21 cells. Reactivity between insect cell expressed gB and anti-HCMV specific antibody in rabbit sera was detected by using fluoroscein isothiocyanate (FITC) conjugated anti-rabbit IgG.

3. Results

3.1. Amino terminal fusion

Plasmids pPV404 and pv404CMV were grown in *E. coli* and the *tac* promoter was induced. The expressed proteins, PV–HBc_{1-183} (a poliovirus fusion protein) and HCMV–HBc_{1-183} were visualised by Coomassie blue staining after SDS–PAGE and compared with uninduced cell extracts (Fig. 2a). Expression of a protein with molecular weight of 24–26 kDa (Fig. 2a, lane 2) was detected in cells harbouring the control plasmid pPV404. A fusion protein corresponding to HCMV–HBc_{1-183} protein could not be detected (Fig. 2a, lane 4). Although further purification was not carried out for the HCMV–HBc_{1-183} protein, examination by electron microscopy revealed relatively few core-like particles (Fig. 2c) compared with control cultures (Fig. 2b). HCMV–HBc_{1-183} fusion protein when analysed by dot blot (results not shown) and western blot immunoassays using a panel of anti-HBc monoclonal antibodies (results only shown for one monoclonal antibody; Fig. 4, lane 4), did not demonstrate any HBcAg reactivity, although the control PV–HBc_{1-183} fusion protein did react with all anti-HBc monoclonal antibodies tested (Fig. 4, lane 3).

3.2. Carboxyl terminal fusion

Gene expression in ptacHpaII–R2 also is driven by the *tac* promoter. The plasmid is engineered so that it contains the initiation codon of the β-galactosidase gene fused to the third codon of HBcAg. The plasmid contains the coding sequence for the first 144 amino acid residues of HBcAg (thereby deleting aa 145–183) and a *Hin*dIII site is present at this position to facilitate ligation to other sequences [9] (Fig. 1). Therefore, a primer pair (CMV1 and CMV2)

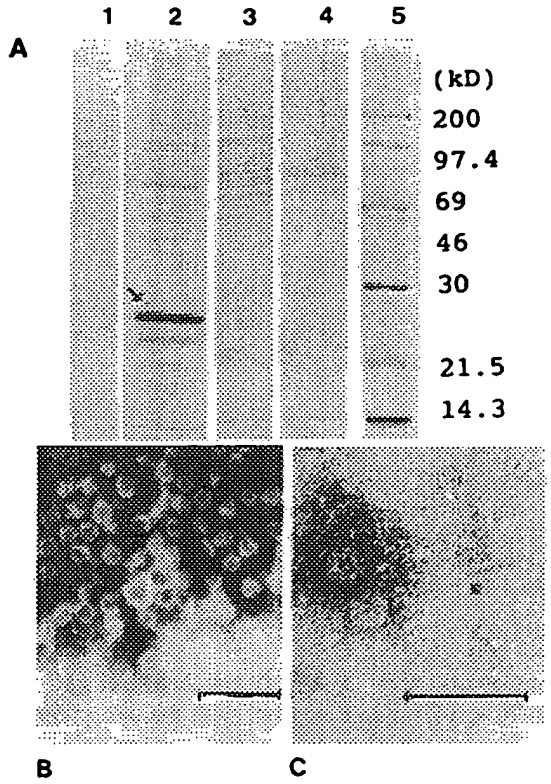


Fig. 2. (A) SDS–PAGE and Coomassie blue staining of proteins expressed after IPTG-induction of cultures harbouring plasmids pPV404 (poliovirus–HBc_{1-183} amino terminal fusion) and pv404CMV (HCMV–HBc_{1-183} amino terminal fusion). Lane 1, PV–HBc_{1-183} pre-IPTG induction; lane 2, PV–HBc_{1-183} post-IPTG induction (band marked with an arrow); lane 3, HCMV–HBc_{1-183} pre-IPTG induction; lane 4, HCMV–HBc_{1-183} post-IPTG induction; lane 5, protein molecular weight markers in kilodaltons (kDa). (B and C) Electron micrographs showing core-like particles in IPTG-induced cultures of cells harbouring plasmids pv404CMV (HCMV–HBc_{1-183}, panel B) and pPV404 (PV–HBc_{1-183}, panel C). Size bar = 100 nm.

containing *Eco*RI and *Bam*HI sites was used to amplify HCMV sequences from p73HCMV. The nucleotide sequence at the junction sites was confirmed and protein expression was induced with IPTG as described in Section 2. Proteins of 18 kDa, representing truncated $HBcAg_{3-144}$, and 22 kDa, corresponding to the HBc_{3-144}–HCMV fusion protein, were visualised by SDS–PAGE/Coomassie blue staining (Fig. 3a). The difference in mobility between the truncated $HBcAg_{3-144}$ and the HBc_{3-144}–HCMV fusion protein is in good agreement with that predicted from the length of amino acid sequence encoded by the HCMV insert. It was observed that some truncated $HBcAg_{3-144}$ and HBc_{3-144}–HCMV protein was also present in the uninduced cell extracts (Fig. 3a, lanes 2 and 4). This could be explained by the relatively low amounts of the lac repressor in this strain of *E. coli*, leading to constitutive expression of the proteins.

The truncated $HBcAg_{3-144}$ and HBc_{3-144}–HCMV proteins were purified by chromatography and ultracentrifugation and visualised by SDS–PAGE and Coomassie blue staining. Yields ranged from 2 to 10 mg/l of culture. Success of the method employed to purify the HBcAg fusion proteins by pelleting using ultracentrifugation indicates that the fusion protein is assembled into core-like particles. The particles were visualised using electron microscopy and resemble recombinant HBcAg particles produced in *E. coli* (Fig. 3b, c).

HBc antigenicity of the HBc_{3-144}–HCMV fusion protein was analysed by dot blot (data not shown), western blot and RIA. The (rabbit) polyclonal anti-HBc antiserum recognised the truncated $HBcAg_{3-144}$ and HBc_{3-144}–HCMV fusion protein in monomeric, dimeric and high molecular weight forms in a western blot (results not shown). However, of a panel of five anti-HBc monoclonal antibodies, only one, RFHBc17, detected HBc_{3-144}–HCMV in a western blot (Fig. 4, lane 2). Three of the monoclonal antibodies detected the protein in a dot blot assay (results not shown), suggesting conformational changes to several exposed HBc epitopes may have resulted during SDS–PAGE. The HBc reactivity of HBc_{3-144}–HCMV also was analysed by a sandwich RIA using a solid phase coated with polyclonal rabbit anti-HBc IgG, showing a dose-response binding to anti-HBc labelled beads similar to that produced by truncated $HBcAg_{3-144}$ (Fig. 5). The signal generated in this antigen capture RIA was high using truncated $HBcAg_{3-144}$ and between 1–100 ng of antigen could be detected. When HBc_{3-144}–HCMV was used, the signal generated was not as high as that with the control and more antigen was required to produce a similar signal to the control antigen (Figs. 6 and 7).

Antigenicity of HBc_{3-144}–HCMV could not be demonstrated using anti-HCMV monoclonal 7-17 or anti-HCMV human polyclonal antibodies in dot blot

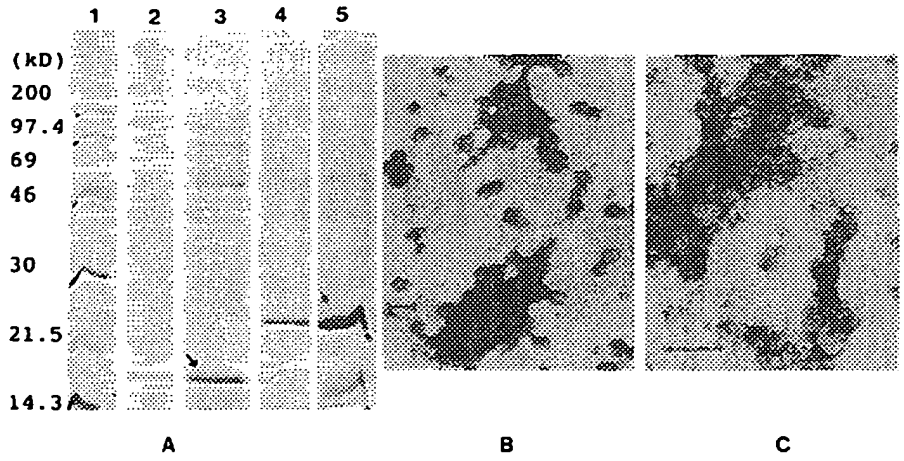


Fig. 3. (A) SDS–PAGE and Coomassie blue staining of proteins expressed after IPTG-induction of cultures harbouring ptacHpaII–R2 (truncated $HBcAg_{3-144}$) and ptacCMV (HBc_{3-144}–HCMV). Lane 1, protein molecular weight markers in kDa; lane 2, truncated $HBcAg_{3-144}$ pre-IPTG induction; lane 3, truncated $HBcAg_{3-144}$ post-IPTG induction (band marked with an arrow); lane 4, HBc_{3-144}–HCMV pre-IPTG induction; lane 5, HBc_{3-144}–HCMV post-IPTG induction (band marked with an arrow). (B and C) Electron micrographs showing core-like particles in IPTG-induced cultures of cells harbouring plasmids ptacHpaII–R2 (truncated $HBcAg_{3-144}$, panel B) and ptacCMV (HBc_{3-144}–HCMV, panel C). Size bar = 50 nm.

and western blot assays. Furthermore, negative results were obtained using RIA in which the capturing solid phase was the same as that used for HBc

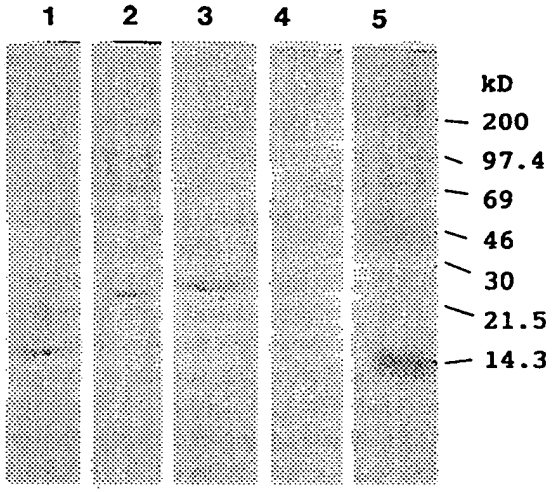


Fig. 4. Western blot analysis of HBc_{3-144}–HCMV and HCMV–HBc_{1-183} using anti-HBc monoclonal antibody RFHBc17. Purified protein samples were separated by 12.5% SDS–PAGE and transferred to a nitrocellulose membrane. HBc reactivity was detected by using polyclonal rabbit anti-HBc serum with the alkaline phosphatase conjugate detection system. Lane 1, truncated $HBcAg_{3-144}$; lane 2, HBc_{3-144}–HCMV; lane 3, PV–HBc_{1-183}; lane 4, HCMV–HBc_{1-183}; lane 5, protein molecular weight markers in kDa.

antigenicity, that is polystyrene beads coated with rabbit anti-HBc antibody but with the exception that the second antibody was either a monoclonal or a polyclonal anti-HCMV antibody. The failure to detect HCMV antigenicity suggests that the HCMV epitope might not be exposed on the surface of the hybrid core particles or have all of the amino acid residues required for conformational integrity.

3.3. Immunisation studies

All the animals mounted an efficient immune response against the fusion protein HBc_{3-144}–HCMV

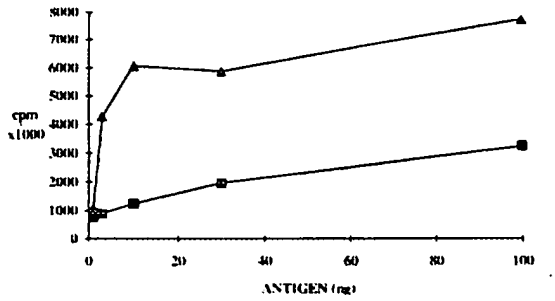


Fig. 5. Sandwich RIA to determine HBc antigenicity of HBc_{3-144}–HCMV (■) and truncated $HBcAg_{3-144}$ (▲).

and produced anti-HBc antibodies, which were detected 10 days after commencing immunisation. A maximal anti-HBc response was achieved at day 40, ten days after the first booster injection and this antibody level was maintained or increased slightly after the second booster injection on day 60 (Fig. 6). The fourth test bleed obtained on day 100 was not analysed for anti-HBc response. Although all the rabbits produced a similar response, the anti-HBc levels in rabbit 732 were higher than the other two rabbits. As one of the rabbits (R730) died from a chest infection on day 65, it was not possible to analyse a third test bleed from this animal.

Although HCMV antigenicity had not been detected so far, immunofluorescence studies confirmed the presence of anti-HCMVgB specific antibodies in the rabbit sera. Strong cytoplasmic immunofluorescence was detected when immune rabbit serum from the third and the fourth test bleeds was incubated with insect cells which express gB. This reactivity was detectable at a one in sixty dilution of the serum. Only weak immunofluorescence that was diffusely spread in the insect cell was seen when preimmune serum was used as a control. Similar non specific fluorescence was noted when immune rabbit serum was tested against uninfected insect cells. These results provide evidence that HCMV-gp58

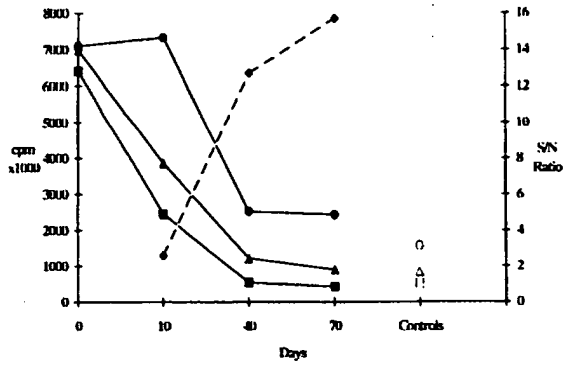


Fig. 6. Induction of anti-HBc antibody in rabbits immunised with HBc_{3-144}–HCMV, determined using a competitive RIA. Rabbits R730, R731 and R732 were immunised with HBc_{3-144}–HCMV on days 0, 30, 60 and 90 and the serum samples were obtained on day 0, 10, 40, 70 and 100. Sera were diluted 1 in 10 (■), 1 in 30 (▲) and 1 in 100 (●) and tested in duplicate for the presence of anti-HBc antibody by competitive RIA. A high titre positive control serum obtained from R86 immunised with HBcAg diluted 1 in 10 (□), 1 in 30 (△) and 1 in 100 (○) and assayed concomitantly. Signal/noise (S/N) ratio is indicated as a broken line.

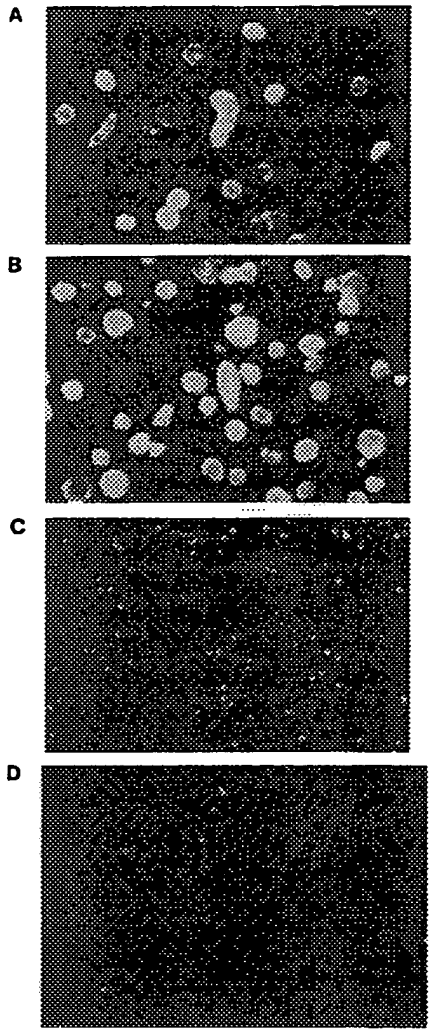


Fig. 7. Detection by immunofluorescence of anti-HCMV specific antibody in rabbit sera. Sf21 cells infected with a recombinant baculovirus expressing HCMVgB were fixed to the glass slide and incubated with immune or preimmune rabbit sera. FITC conjugated anti-rabbit antibody was added to detect any reactivity between HCMVgB and rabbit sera. (A) Positive control: anti-HCMVgB-specific antibody (C23) against Sf21 cells expressing HCMVgB. (B) Serum from R731 (fourth test bleed) against Sf21 cells expressing HCMVgB. (C) Preimmune serum from R731 against Sf21 cells expressing HCMVgB infected. (D) Serum from R731 against uninfected Sf21 cells.

epitope was immunogenic when presented to the immune system with HBcAg.

4. Discussion

HBcAg fusion proteins have been expressed in *E. coli* and purified on the basis of their assembly into core-like particles [2,9]. Although the same method was employed here, the results suggest that amino terminal fusion protein HCMV–HBc_{1-183} either was not expressed or was expressed only in very low quantities, as the protein could not be detected by Coomassie blue staining, dot blot immunoassays or western blotting. In a study by Shiau [20], inability to purify HBcAg fusion proteins containing sequences encoding the S, preS1 and preS2 regions of HBV, and carrying inserts at both amino and carboxyl termini of truncated HBcAg, was attributed to protein aggregation due to some inherent properties of the foreign epitope. Utz et al. [21] reported that a stretch of amino acids in HCMV–gp58 that bind to the HCMV neutralising antibody 7-17 are hydrophobic and, indeed, this characteristic has been displayed in hydrophobicity profiles [22], generated for the amino and carboxyl terminal fusions with HBcAg. The hydrophobic nature of the gp58 epitope could explain the poor solubility of the recombinant polypeptide. Although HCMV–HBc_{1-183} could not be purified for immunological study, the observation of particles by electron microscopy suggested that the fusion protein is capable of assembling into core particles.

The carboxyl terminal fusion HBc_{3-144}–HCMV and the control, truncated HBcAg ($HBcAg_{3-144}$) display a similar HBc reactivity in several assays, indicating that fusion of foreign sequences to the carboxyl terminus of HBcAg does not abrogate HBc antigenicity. In western blotting, only one anti-HBc monoclonal (RFHBc17) reacted with HBc_{3-144}–HCMV and the control proteins. The same solid support was used for binding the fusion protein in western and dot blot formats. In western blotting, however, the protein would probably not be in the native form, since it was denatured by treatment with ionic detergent and boiling under reducing conditions. Although electrophoretic transfer of the protein to the nitrocellulose membrane could remove the detergent and the reducing agent, the covalent forces which result in binding of the protein to the membrane may prevent complete renaturation of the polypeptide. Therefore, it is likely that western blotting would favour linear determinants [20]. This suggests that monoclonal RFHBc17 recognises a linear determinant whereas the other monoclonal antibodies may recognise a conformational determinant/s present only on the native HBcAg. This hypothesis is supported by the fact that the antibody was raised against HBcAg purified from infected chimpanzee liver and following treatment with 0.1% SDS and 0.1% 2-mercaptoethanol at 37°C for 2 h [15]. In the antigen capture RIA using rabbit polyclonal anti-HBc antibody, HBc_{3-144}–HCMV displayed a similar antigenicity to the control truncated $HBcAg_{3-144}$. Although the signal generated was weaker for HBc_{3-144}–HCMV than for the control, these results are in accordance with the previous findings that HBc antigenicity is retained in carboxyl terminal fusions [9,10,13].

The HCMV–gp58 epitope was found not to be antigenic in either dot blot immunoassays or SDS–PAGE immunoblotting using anti-HCMV monoclonal and (human) polyclonal antibodies. The absence of reactivity with anti-HCMV antibodies raises the possibility that the HCMV epitope might not have been expressed at all. However, the results obtained by sequencing both fusion constructs indicate that there was no stop codon or disruption of the reading frame that could have prevented the expression of the fusion proteins. Instability or misfolding of a hybrid polypeptide has been the main impediment to the development of generally applicable strategies for chimeric particle formation [23]. A similar problem may have arisen in this study due to presence of hydrophobic domains in the gp58 epitope. This suggestion is strengthened by the finding in this study that gp58 antigenicity was not preserved and could have resulted from misfolding of the hybrid polypeptide in such a way that HBcAg B-cell epitopes were exposed on the surface of the particle but gp58 epitopes were masked, being sequestered inside the particle. The apparent loss of gp58 antigenicity observed for both fusion proteins supports the hypothesis of conformational change attributable to the hydrophobic nature of this region of gp58. Thus, it is possible that addition of a hydrophobic

amino acids has altered the topology molecule in a way that inhibits its assembly into a hybrid core particle. It also is interesting to note that more recent data have shown that the immunodominant region AD-1 of gp58, which contains the recognition site for monoclonal antibody 7-17, comprises around seventy amino acid residues [18]. Thus, conformational integrity required for recognition by 7-17 may be lacking in the fifty residue HCMV peptide sequence used in these experiments.

The HBc and HCMV immunogenicity of HBc_{3-144}–HCMV was determined in terms of antibody production in rabbits. Immunisation of rabbits with HBc_{3-144}–HCMV produced high levels of HBcAg-specific antibodies, indicating that fusion of the HCMV–gp58 epitope to the carboxyl terminus of HBcAg had not altered the immunogenicity of HBcAg. Although all the immunised rabbits mounted a strong immune response to the core component of the HBc_{3-144}–HCMV fusion protein, determined using RIA, the antibody response to the HCMV epitope could not be analysed or compared because of the lack of availability of appropriate antigens that could be used in RIA. Therefore, anti-HCMV antibodies initially were analysed using an Abbott CMV latex agglutination test but the results were non-specific (results not shown). This was followed by immunofluorescence using insect cells infected with a recombinant baculovirus expressing HCMV gp58. The presence of anti-HCMV specific antibodies was confirmed using this technique and immunofluorescence produced by the immune rabbit sera provides the evidence that the HCMV epitope is immunogenic, although these antibodies were of low titre.

In conclusion, two separate fusions were constructed between HCMV–gp58 and the amino and carboxyl termini of HBcAg. When expressed in *E. coli*, HBc antigenicity was retained only by the HBcAg carboxyl terminal fusion and HCMV antigenicity could not be detected for either fusion. The immunogenicity of the HBcAg carboxyl terminal fusion was determined by antibody production in rabbits. The results demonstrate that immunisation of rabbits with the fusion protein resulted in generation of HBcAg-specific and HCMV-specific antibodies. The HBcAg-specific antibodies were produced only a few days after the primary immunisation whereas anti-HCMV response was delayed. Nevertheless this indicates that HBcAg can serve as a carrier for presentation of HCMV–gp58 epitope to the immune system emphasising the potential value of this epitope, perhaps in a larger form, as a candidate for a subunit HCMV vaccine.

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